

TECH CENTER 1600/2900

SEP 09 2002

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**p7.5tk**

**7.5 k promoter**

**Not I**

**Apai**

GGCCAAAATTGAAAAAAGTACTATTATTGCACAGCGGCCGCGGGC CCG GCC AAC GCC GGA

Met Gly Pro Ala Ala Asn Gly Gly

## tk coding sequence

**pE/Ltk**

**synthetic E/L promoter**

**Not!**

## Apai

GGCCAAAATTGAATTTTATTTTTTTTGGATATATAAGCGCCCGCATG GGC CCG GCC GCC AAC GGC AAC GGC GGA

Met Gly Pro Ala Ala Asn Gly Gly

## tk coding sequence

**FIG. 1**



1. p7.5tk

7.5K PROMOTER NOTI APAI  
 5'- GGCCAAAATTGAAAACCTAGATCTATTATTGACACGGCGCCGCCATGGGCCCCGGCC -3'

2. p7.5/ATG0/tk

7.5K PROMOTER NOTI BAMHI SMAI PSTI  
 5'- GGCCAAAATTGAAAACCTAGATCTATTATTGACACGGCGCCGGCGGATCCCCGGGCTGCAGGAA

TRANSLATION TRANSCRIPTION  
 SALI STOP CODONS STOP SIGNAL  
 TTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCTAACTAAATTTGTTTGT

APAI

GGGCCCCGGCC -3'

FIG. 2



# START

## 7.5K PROMOTER

NOTI

**CODON BAMHI SMAI PSTI**

5'-GGCCAAAATTGAAAACTAGATCTATTATTGCACGGCGCCGCGCCATGgtGgATCCCCCGGGCTGCAGGAA

TRANSCRIPTION  
TRANSLATION

## TRANSCRIPTION

**SALI**

## STOP CODONS

TTTCGATATCAAGCTTATCGATACCGTTCGACCTCGAGGGGGGGCCTAACTAACTAATTTGTTTGTGT

**APAI**

GGGCCGGCC-3'

**FIG. 2 (cont.)**

Appl. No. 08/935,377; Filed: September 22, 1997  
 Dkt. No. 1821.0010000/EKS/HCC; Group Art Unit: 18  
 Inventors: Maurice ZAUDERER; Tel: 202/371-2600  
 Title: Methods for Selecting Polynucleotides Encoding T Cell  
 Epitopes (as amended)



4. p7.5/ATG2/tk

5'- GGCCAAAATTGAAAACTAGATCTATTATTGACGGCGCGCCCATGAGTGATCCCCGGGCTGCAGGAA

START

7.5K PROMOTER

NOTI

CODON BAMHI SMAI

PSTI

TRANSCRIPTION

TRANSLATION

SALI

STOP CODONS

STOP SIGNAL

TTCGATATCAAGCTTATCGATACCGTTCGACCTCGAGGGGGGGCCTAACTAAATTTGTTTTTGT

APAI

GGGCCCCGGCC -3'

FIG. 2 (cont.)



5. p7.5/ATG3/tk

START

7.5K PROMOTER

NOTI

CODON

BAMHI SMAI

PSTI

5'- GGCCAAAAATTGAAAAAACTAGATCTATTATTGCACGGGGCCGCCATGACGTGGATCCCCGGGGCTGCAGGAA

TRANSLATION TRANSCRIPTION

SALI

STOP CODONS

STOP SIGNAL

TTCGATATCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCTAACTAAATTTGTTTGT

APAI

GGGCCCGGCC -3'

*FIG. 2 (cont.)*

Tumor cDNA Ligated to Adaptor 2

I. Tumor cDNA Ligated to Adaptor 1

Parental cDNA

First Hybridization

III. Second Hybridization  
 + fresh denatured parental cDNA

IV. Fill in with  
 KlenTaq Polymerase

PCR  
 V. AMPLIFICATION

Linear amp

No amp\*

No amp

EXPONENTIAL  
 AMP

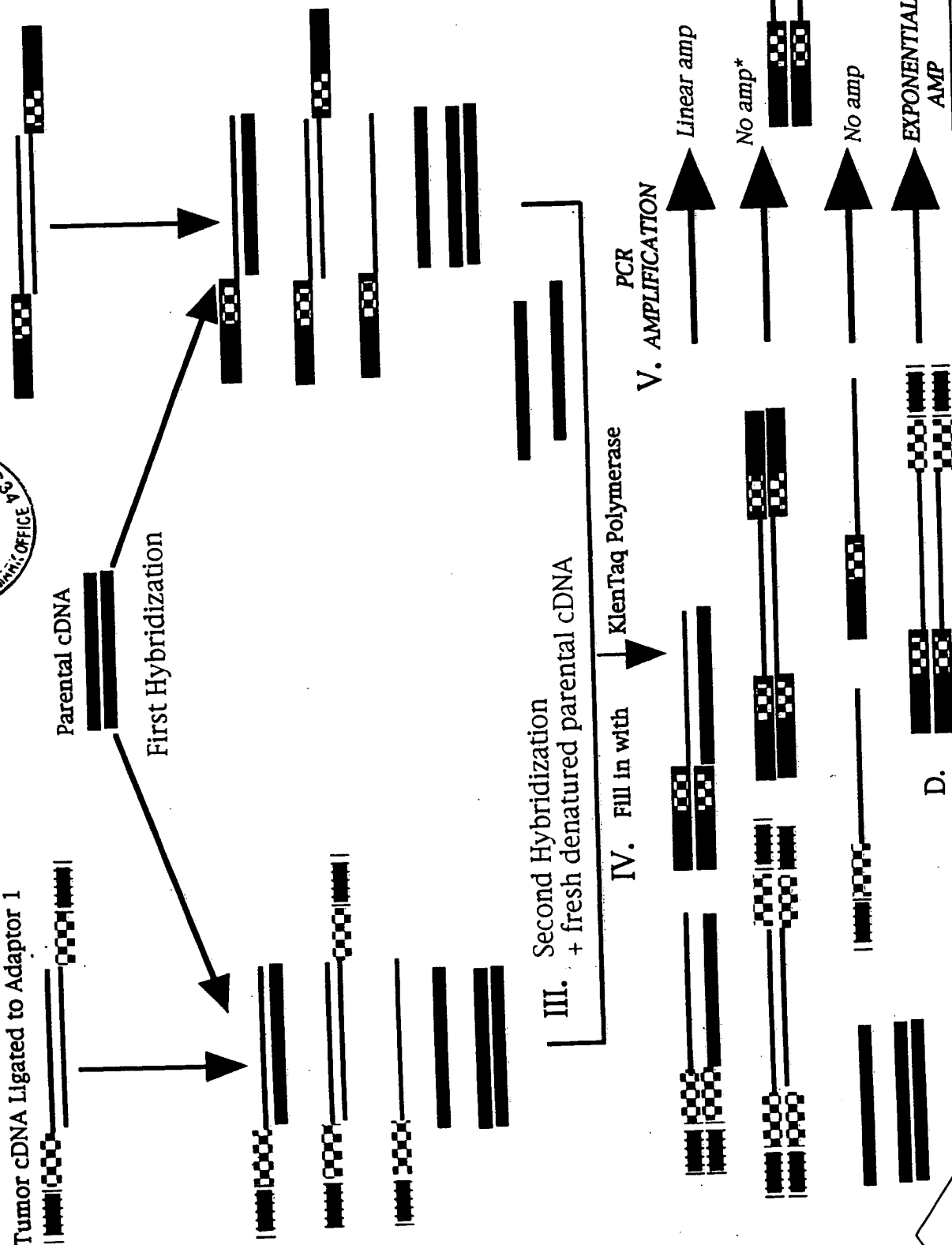
A.

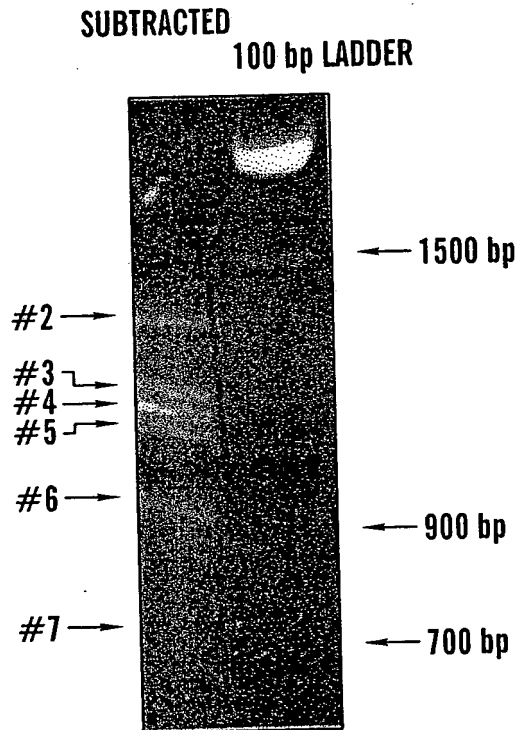
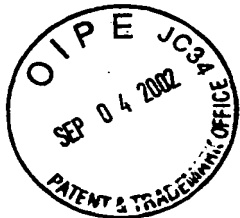
B.

C.

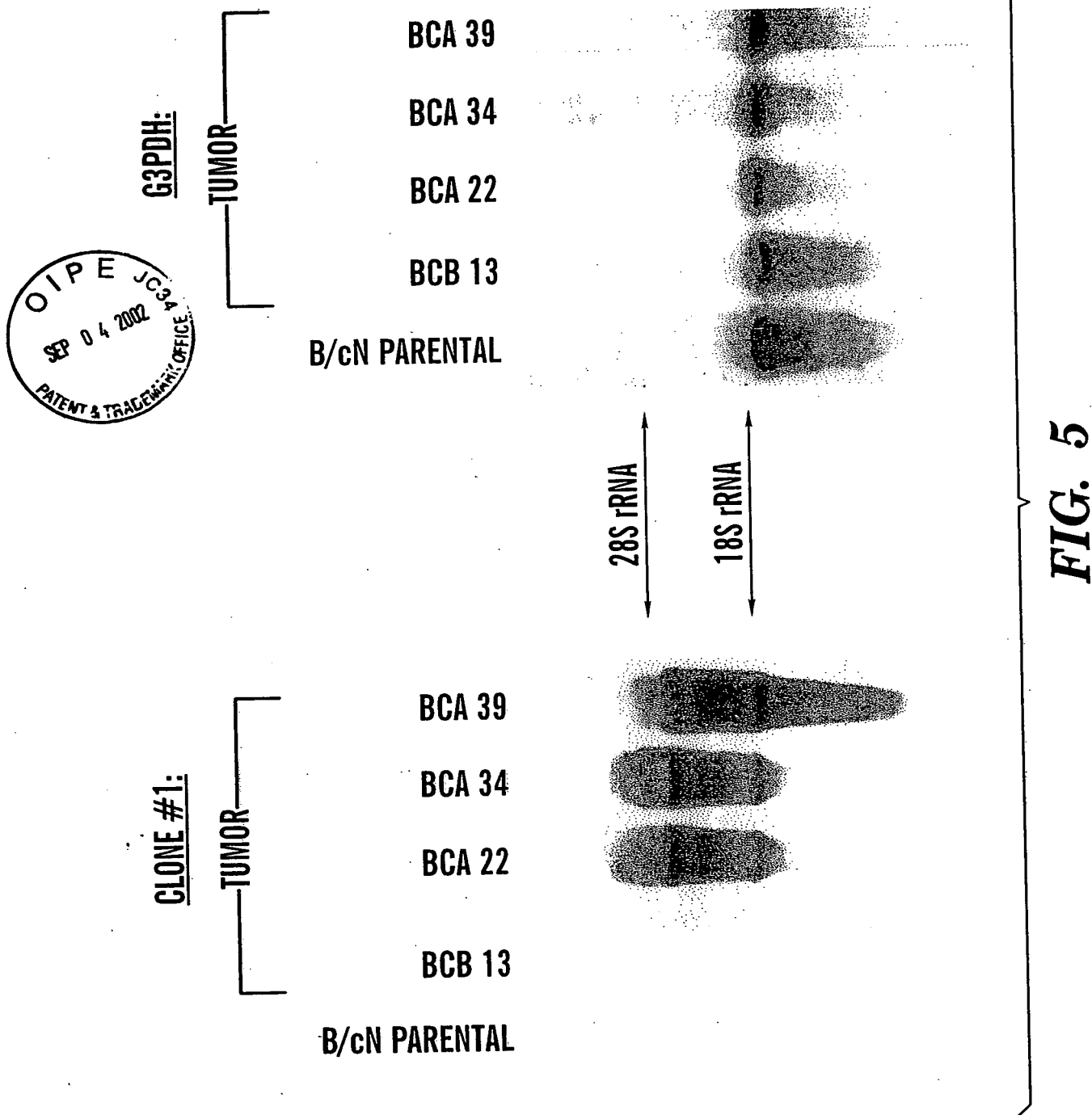
D.

FIG. 3





**FIG. 4**





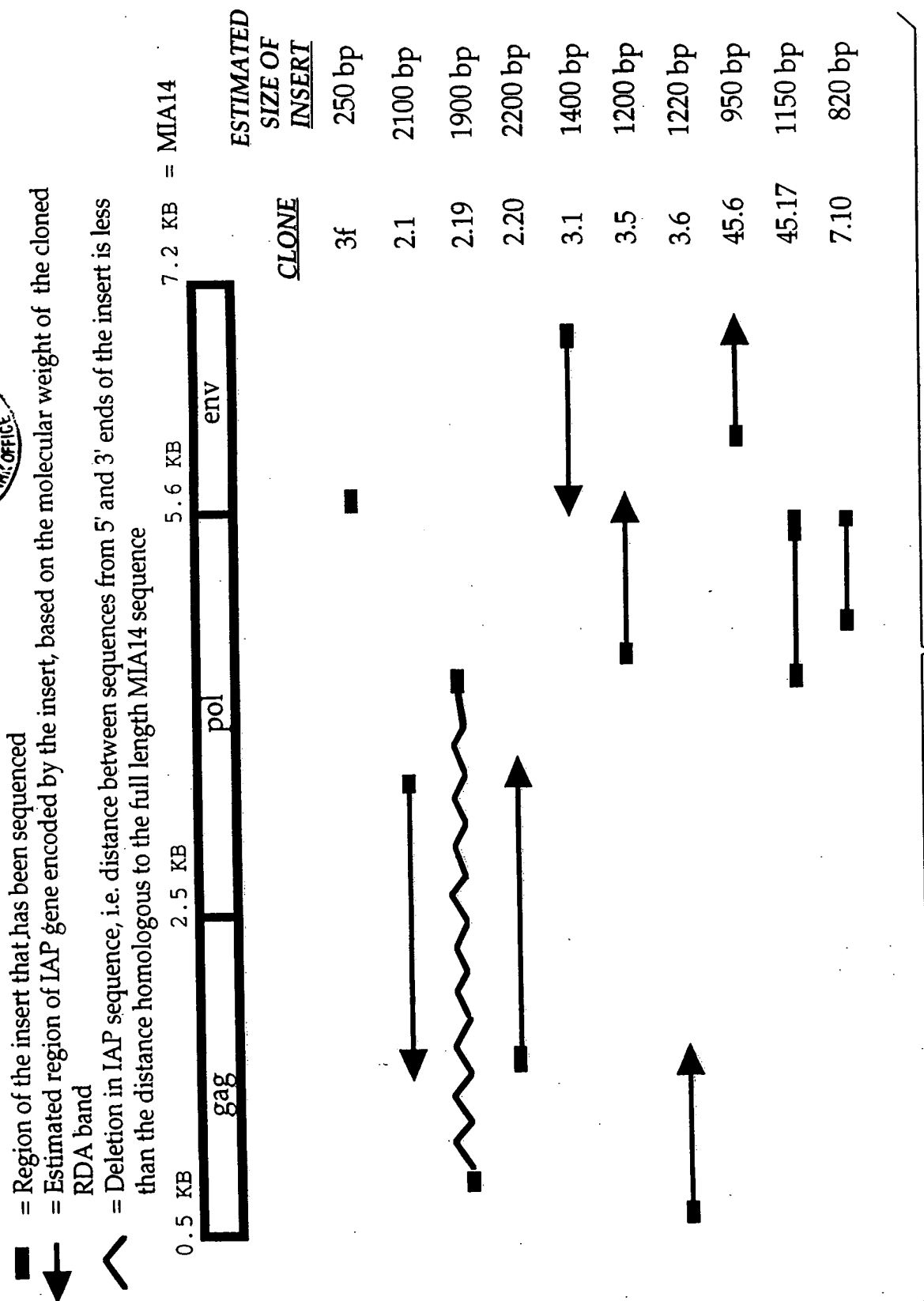
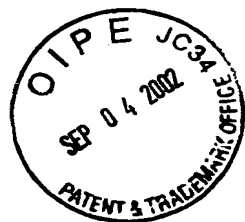
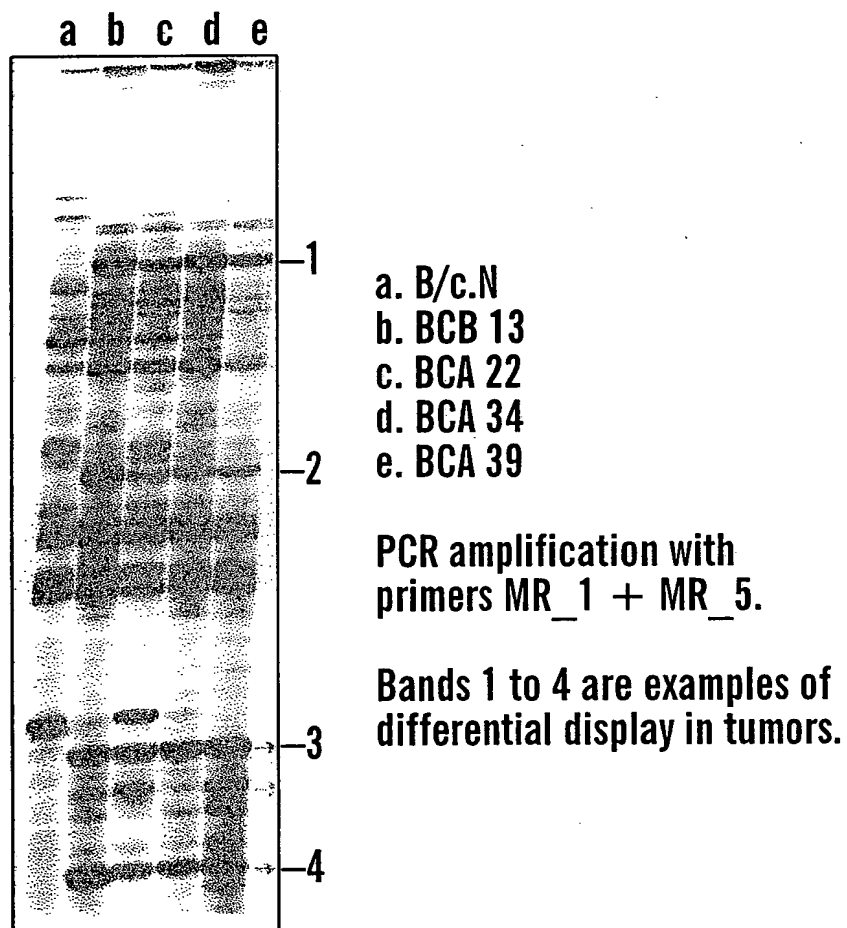
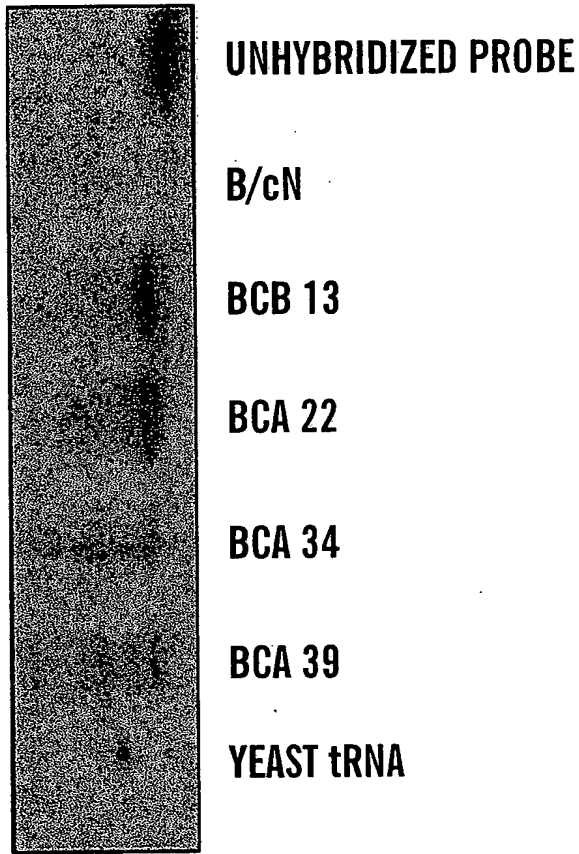
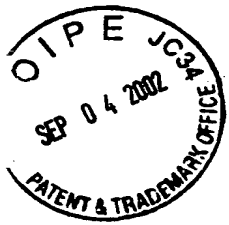


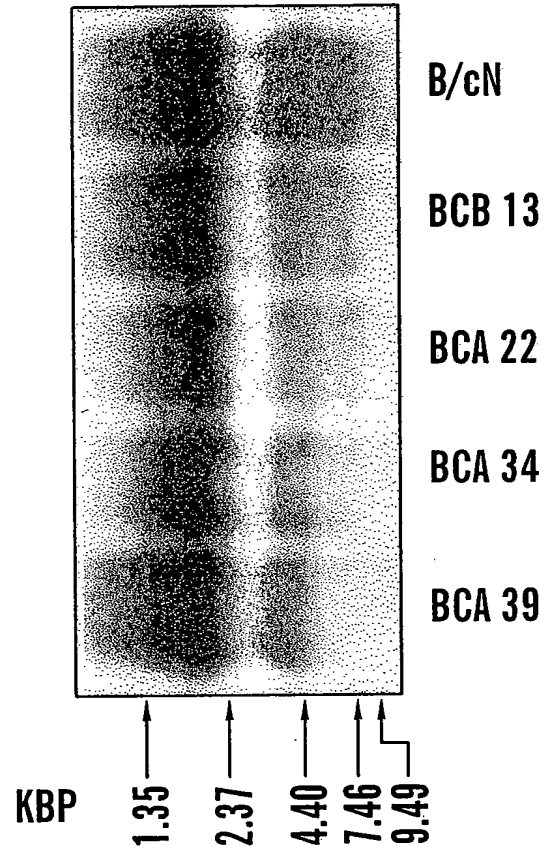
FIG. 6



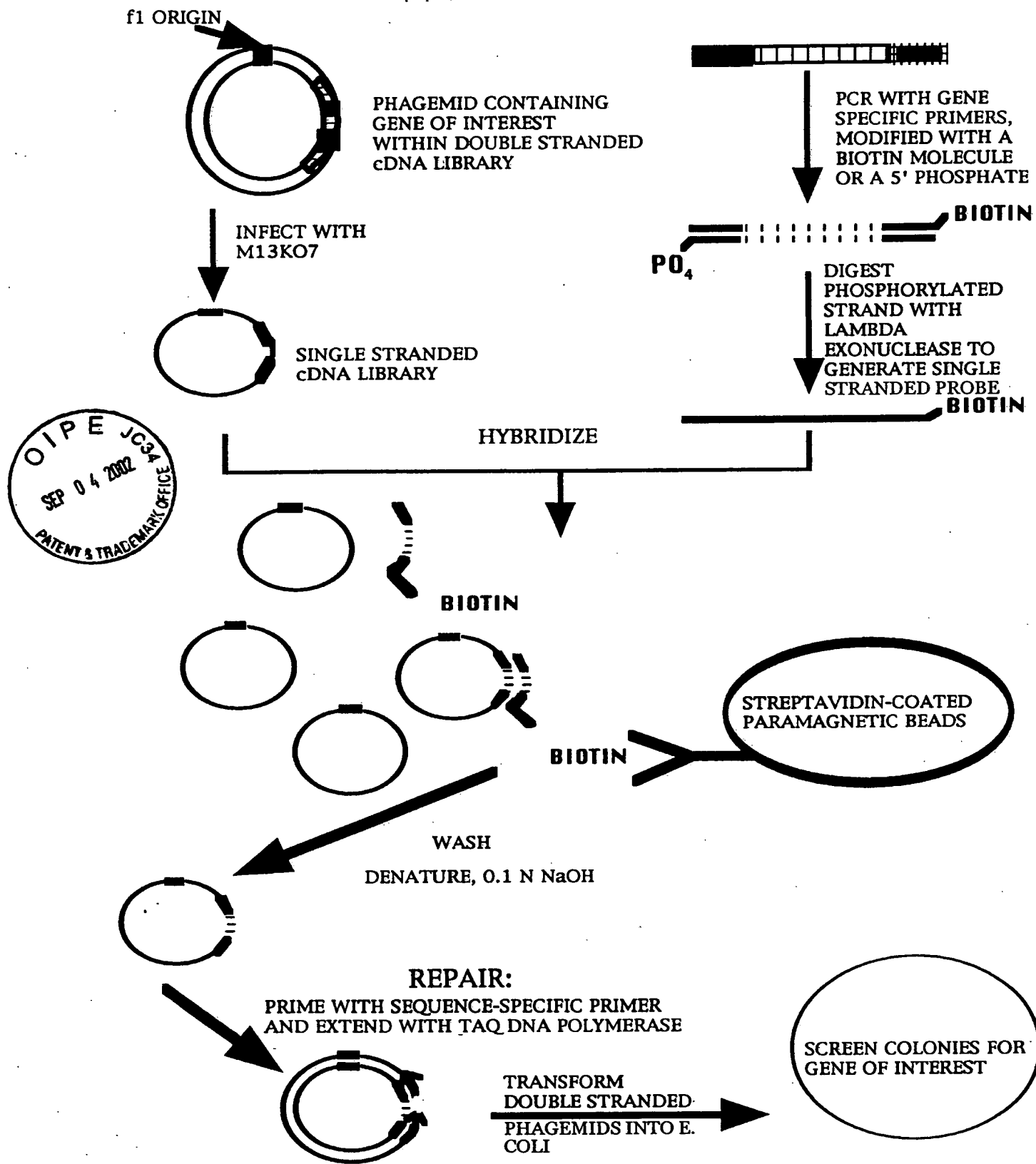
**FIG. 7**



**FIG. 8A**



**FIG. 8B**



**FIG. 9**

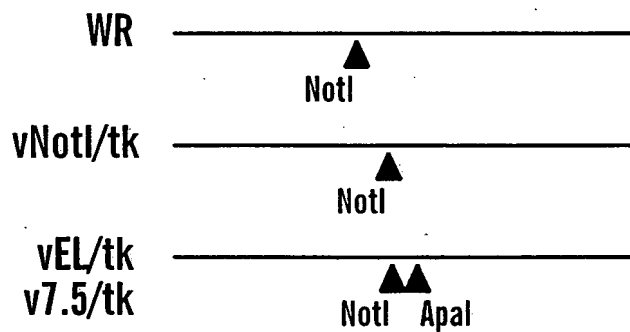
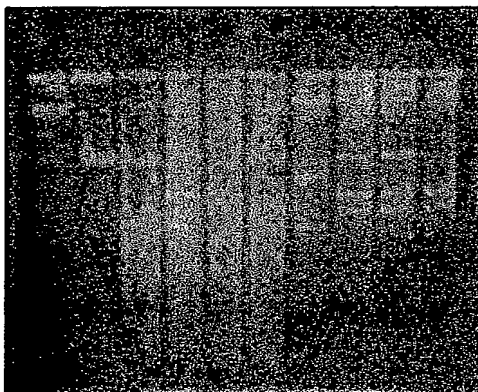
Appl. No. 08/935,377; Filed: September 22, 1997  
 Dkt. No. 1821.0010000/EKS/HCC; Group Art Unit: 1644  
 Inventors: Maurice ZAUDERER; Tel: 202/371-2600  
 Title: Methods for Selecting Polynucleotides Encoding T Cell  
 Epitopes (as amended)



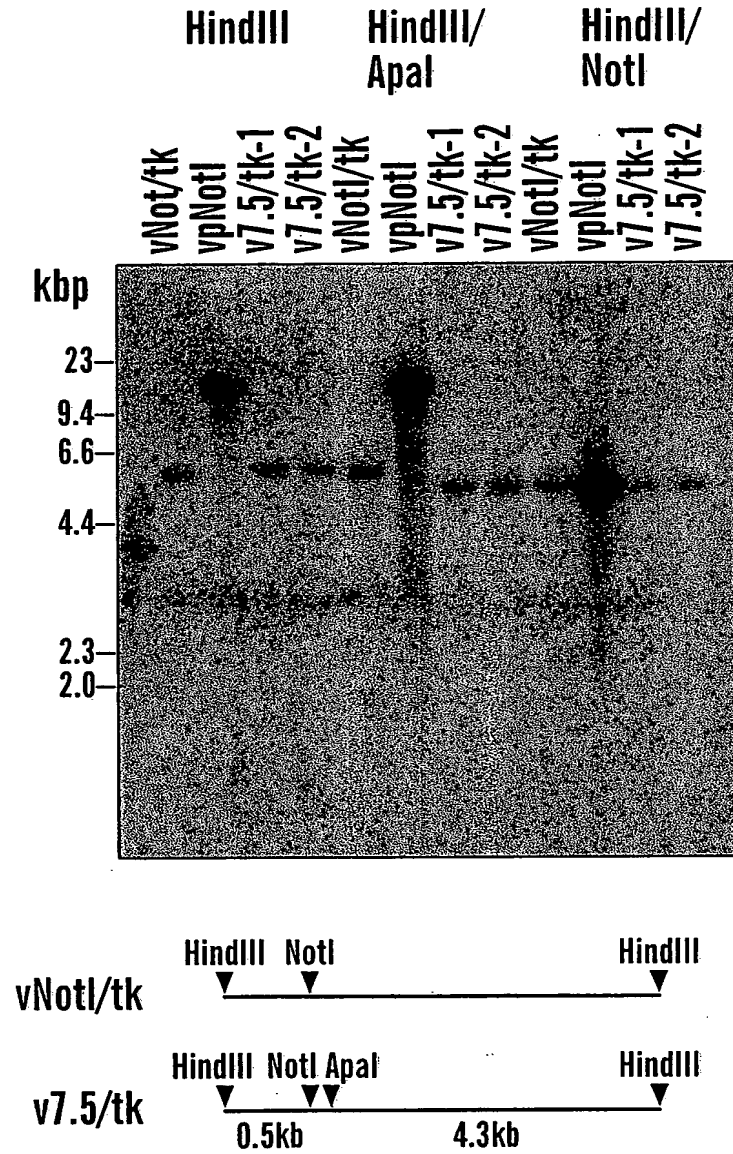
Apal

NotI

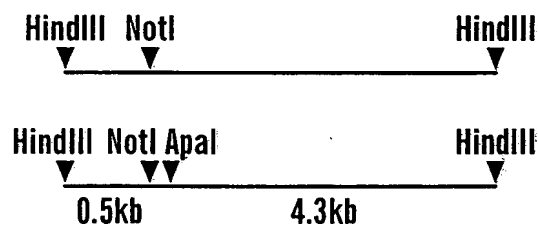
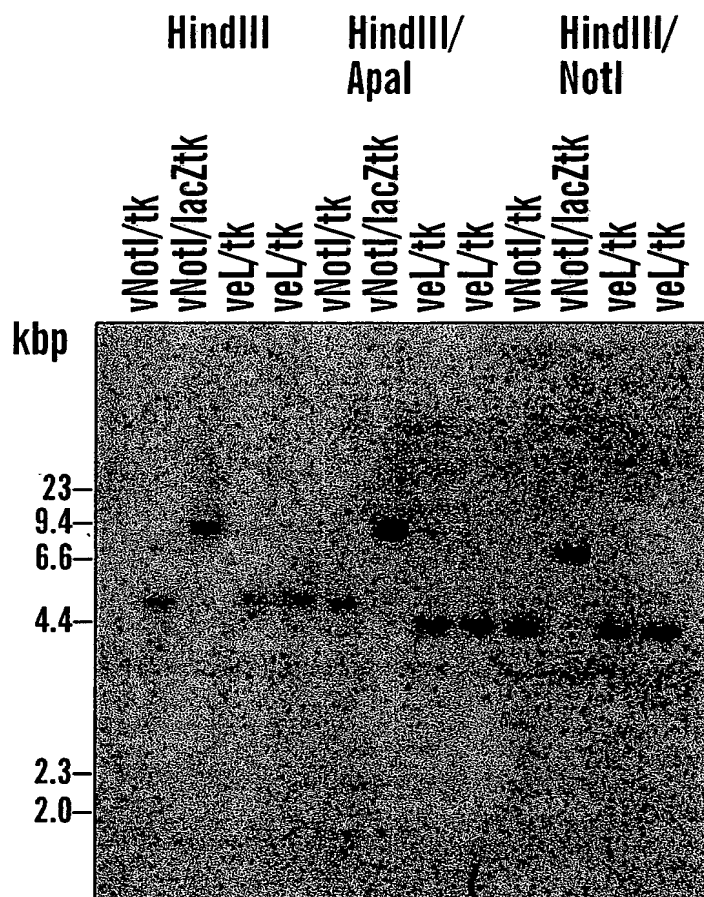
$\lambda$  WR WR vEL v7.5 vNot/tk  
 WR vEL v7.5 vNot/tk



**FIG. 10**

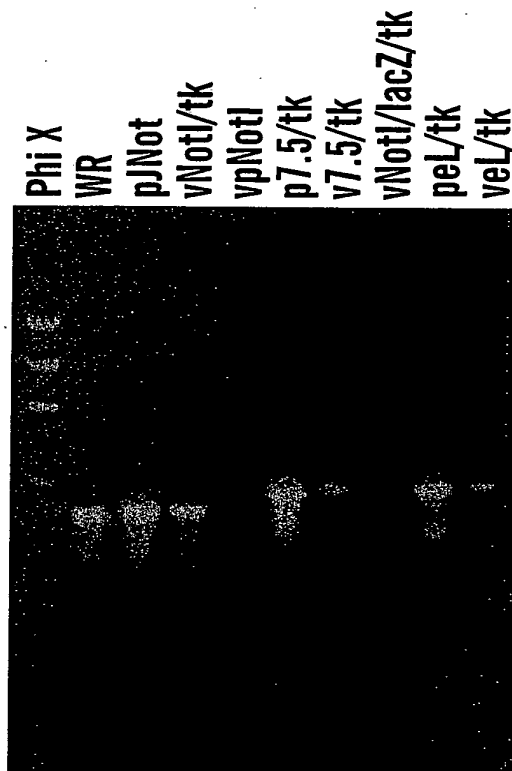


**FIG. 11A**



**FIG. 11B**

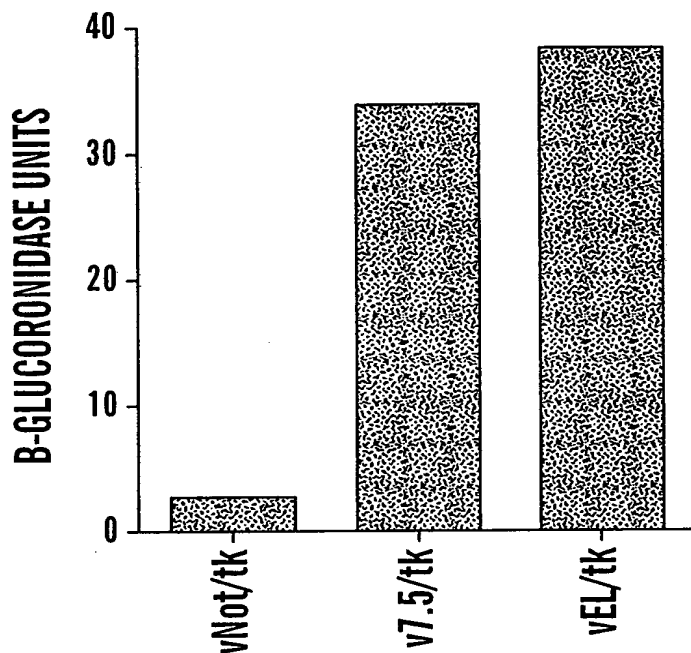
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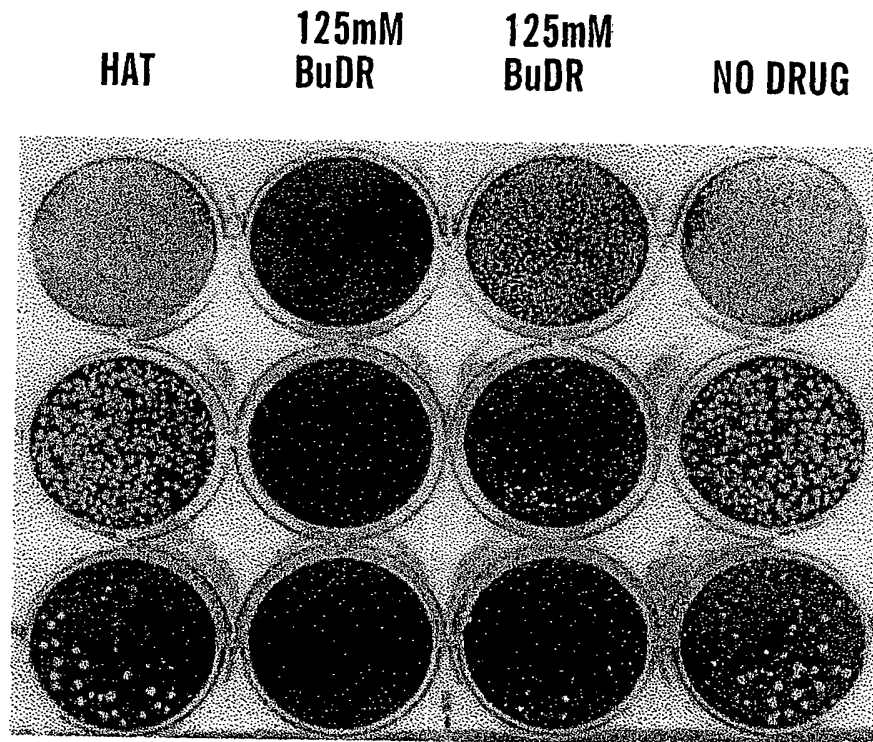
**FIG. 12**



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Title: Methods for Selecting Polynucleotides Encoding T Cell  
Epitopes (as amended)



**FIG. 13**



**FIG. 14**